SECTION 6

SAMPLE ANALYSIS TECHNIQUES

6.1 Introduction

6.1.1 One of the major concerns of USEPA, other federal, state and private agencies or laboratories is to describe water quality and habitat quality in terms which are easily understood by the nonbiologist. Fish studies frequently include the number of specimens captured per unit area or unit time. Also, the fish can be measured, weighted, aged, and sexed to provide comparative data between populations in different habitats. The purpose of this section is not to recommend one particular data evaluation method, but to point out a number of more common methods. Some of these methods may not be applicable to every stream, lake, or water body in the United States. Methods, techniques, and biological criteria used to study fisheries biology and to analyze fisheries data are described in this manual, elsewhere in Bagenal (1978), Lager (1956, 1978), Carlander (1969), Everhart et al. (1975), Gulland (1983), Nielsen and Johnson (1983), Schreck and Moyle (1990), USEPA (1990, 1991), and also in other current literature. To supplement the statistics and data evaluation methods in this section and for additional biometrics, consult the statistical references listed in Section 1, Introduction, Subsection 1.16.1. For other multivariate analyses and other techniques to relate distribution to environmental variables and gradients, confer with Matthews (1985), Matthews and Robison (1988), Mayden (1985; 1988), and McAllister et al. (1986).

6.1.2 Water quality and habitat quality are reflected in the species composition and diversity, population density and biomass, and physiological condition of indigenous communities of aquatic organisms, including fish. A number of data interpretation methods have been developed based on these community characteristics to indicate the health and water quality of the aquatic environment, the degree of habitat degradation, and also to simplify communication problems regarding management decisions.

6.2 Data Recording

6.2.1 The sample records should include collection number, name of water body, date, locality, names of sample collectors, and other pertinent information associated with the sample. Make adequate field notes for each collection. Use water-proof ink and paper to ensure a permanent record. Place the label (Figure 1; also see Section 2, Quality Assurance and Quality Control; Section 5, Fish Specimen Processing) inside the container with the specimens only when fixing or preserving fish for physical examination (**Note: do not place the label with fish if they are to be chemically analyzed.**) and have the label bear the same number or designation as the field notes, including the locality, date, and collector's name. Place a numbered tag on the outside of the container to make it easier to find a particular collection. Place any detailed observations about a collection on the field data sheet (see Section 4, Sample Collection for Analysis of Structure and Function of Fish Communities and Section 8, Fish Bioassessment Protocols for

Use in Streams and Rivers for examples of field data sheets). Record fishery catch data in standard units such as number or weight per area or unit of effort. Use the metric system for length and weight measurements. Designate any chemical analyses to be performed, e.g., toxaphene analysis.

6.3 Fish Identification

6.3.1 Proper identification of fish to species level is mandatory in analysis of the data for water quality interpretation. A list of regional and national references for fish identification is located in Section 8, Fish Bioassessment Protocols for Use in Streams and Rivers; Section 12, Fisheries Bibliography. Assistance in confirming questionable identification is available from State, Federal, and university fishery biologists or ichthyologists. In the Quality Assurance Project Plan (see Section 2, Quality Assurance and Quality Control), key(s) used for fish identification should be specified.

Collection No. ____________________

Project ____________________

Location ____________________

____

Date ________ Time ________ Mile ________

Sampling Device ____________________

Collected by ____________________

Observations ____________________

____

Preservation(s) ____________________

Figure 1. Example of fish sample label information for preserved specimen container.

6.4 Species Composition (Richness)

6.4.1 A list of species can be compiled using any sampling device, technique, or combinations of the two. The method used should not select against one or more species. Also, sampling effort should be thorough enough so that all species are collected from the study area, and the sampling should be conducted several times during the year to include seasonal species. The calculations for percent species composition in a sample is:

$$= \frac{\text{Number of individuals of a given species}}{\text{Total number of all fish collected}} \quad X\ 100.$$

6.5 Length and Weight

6.5.1 Rate of change in length of fish, length frequency distribution, and weight of fish are important attributes of fish populations. These measurements can provide an estimation in growth, standing crop, and production of fish in surface waters.

6.5.1.1 Three length measurements as described by Lagler (1978) are sometimes used in monitoring studies, but total length is used most often. The three length measurements (Figure 2) are standard length, fork length, and total length. Standard length of fish is measured from its most anterior extremity (mouth closed) to the hidden base of the caudal fin rays, where a groove forms naturally when the tail is bent from side to side. Fork length is measured from the most anterior extremity of the fish to the notch in the center of the tail. It is the center of the fin when the tail is not forked. Total length is the greatest length of the fish from the anterior most (mouth closed) and caudal rays squeezed together to give the maximum length measurement. For fish with a forked tail, the two lobes are squeezed together to give a maximum length. If the lobes are unequal, the longer lobe is used.

6.5.1.2 A fish measuring board is commonly used to measure length. Fish measuring boards contain a graduated scale and is usually made of wood or plastic. Lagler (1978) identifies and discusses factors that can cause possible errors and inconsistency in taking length measurements. When taking fish measurements, standard procedures should be written so that the measurements are done the same way if different individuals are involved in this procedure.

6.5.1.3 Measurement of fish weight is taken with an accurate scale that can be used in field studies. Lagler (1978) indicated that precision in weight measurements is not possible because of variation in the amount of stomach contents and the amount of water engulfed at capture of the fish. The weights of live and preserved specimens are not comparable because the percentage of shrinkage is unknown.

6.5.1.4 Additional information on length, weight, and associated structural indices are discussed in Anderson and Gutreuter (1983).

6.6 Age, Growth, and Condition

6.6.1 Changes in water quality can, at times, be detected by studying the age, growth, and condition of fishes taken from a body of water. These studies require extensive knowledge of the life histories of fish and of the area being studied, experience in aging fish, sufficient time and manpower to adequately sample and analyze the data, and sufficient age, growth, and condition historical data for comparison.

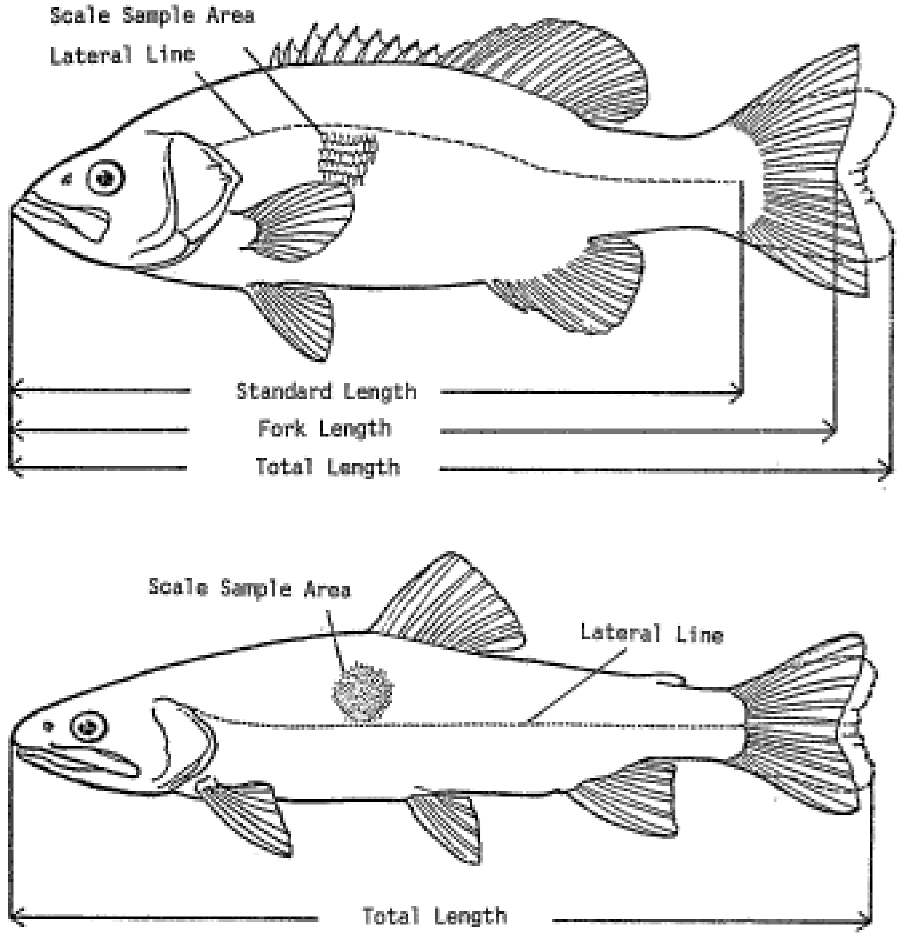

Figure 2. Fish measurements (using a fish measuring board) and scale sampling areas. A. spiny-rayed fish. B. soft-rayed fish. Total length measurement requires compressed tail to give maximum elongation. Modified from Lagler (1956).

6.6.2 A problem in using fish for any type of study is their high mobility. However, Gerking (1959) indicated that many species are relatively sedentary in summer. Depending on the species, there may be no practical way to determine with a first time visit how long an individual fish has been in a given area. Any changes detected in age, growth, or condition are not necessarily attributable to conditions prevailing at the capture site. Some information on fish movement may be obtained from previous State or Federal studies. Only a carefully planned, long-term study may provide beneficial data, and only if used in conjunction with other biological, physical, and chemical data, e.g,, benthic invertebrates (macroinvertebrates), periphyton, water flow, habitat, and water chemistry.

6.6.3 The methods most commonly used in studying the age and growth of fishes are: (1) length-frequency, (2) annulus formations in hard parts, such as otolith, bone, spine rays, and scales.

6.6.3.1 The knowledge of the age and rate of growth of fish is extremely useful in fishery management. The processes of determining fish age and assessing fish growth rates are different, but they are closely related and are usually done at the same time. Table 1 was compiled by the Institute for Fisheries Research, the University of Michigan, Ann Arbor, Michigan from samples taken of Michigan fish during a period of approximately 30 years. The samples were collected mostly during the summer months but all months of the year are represented. Variations occur among states in sample size according to species and age groups, and some averages are more reliable than others. Busacker et al. (1990) discuss various techniques that are used in the study of fish growth, and they provide guidance to the appropriate uses of specific growth methods.

6.7 Length-Frequency Method

6.7.1 The length-frequency method for making age determinations is based on the assumption that fish increase in size with age. When the number of fish per length is plotted on graph paper for a given species if comparing a population. Peaks generally appear for each age group.

6.7.2 For this method to provide meaningful data it is important that the following criteria be met during sampling: (1) the fish must be collected over a short period; (2) large numbers must be obtained, including fish of all sizes; (3) the affected area and a control (unaffected) area must be sampled simultaneously within the same time frame.

6.7.3 For some studies, the length-frequency method may be of limited value because: (1) it is considered not reliable in aging fish beyond their second or third growing season (2) acquiring a large number of fish generally requires several experienced field biologists utilizing different sampling techniques.

6.8 Length-Age Conversion Method

6.8.1 In certain studies, it may be desirable to know the age of fish of a given length (e.g., selection data are normally in terms of length, but for incorporation in yield equations need to be expressed in terms of age.) Length can be converted to age (Gulland, 1983) by fitting all the observed data of mean length at age to a growth equation, such as the von Bertalanffy equation.

$$l_t = L_\infty \, [1 - e^{-K(t-t_0)}]$$

6.8.2 To calculate age (t) in terms of length (l), divide both sides by L_∞, and subtract from unity, resulting in

$$\frac{L_\infty - l_t}{L_\infty} = e^{-K(t-t_0)}$$

taking natural logs of both sides gives

$$\log_e \frac{L_\infty - l_t}{L_\infty} = -K(t-t_0)$$

therefore,

$$t = \frac{1}{K} \log_e \frac{L_\infty}{L_\infty - l_t} + t_0$$

where:

t = age (present)

l = length of individual specimens (length at time (t))

L_∞ = maximum length expected for a particular species

t_0 = the age at which the fish would be zero size

r = growth rate constant

TABLE 1. AVERAGE TOTAL LENGTHS IN INCHES FOR EACH AGE GROUP OF SEVERAL FISHES IN MICHIGAN[1]

Species	Age Group													
	0	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Bluegill	2.3	3.4	4.4	5.5	6.4	7.0	7.5	7.9	8.6	8.8	9.1	9.8	9.7	...
Pumpkinseed	2.8	3.3	4.4	5.2	5.9	6.4	7.9	7.3	7.8	7.4	8.1	9.8	...	...
Black Crappie	3.6	5.1	6.8	8.2	9.0	9.5	10.6	10.9	11.8	12.2	...	...	...	...
Rock bass	1.5	3.1	4.5	5.6	6.5	7.4	8.2	8.9	9.6	9.9	10.1	11.6	11.7	...
Warmouth	....	3.1	4.4	5.2	5.5	6.2	6.7	6.9	6.6	7.5	7.3	...	...	...
Green sunfish	...	3.0	3.9	4.7	5.1	5.7	5.7	5.0	...	...	...	...	...	...
Largemouth bass	3.6	6.1	8.6	10.6	12.2	13.6	15.1	16.7	17.7	18.8	19.8	19.6	20.8	...
Smallmouth bass	3.4	6.1	9.2	11.3	13.3	14.9	15.7	16.8	17.5	18.5	19.2	...	19.2	...
Yellow perch	3.1	4.6	6.1	7.0	8.0	9.0	9.9	10.7	11.3	11.8	12.3	12.3	13.9	13.2
Walleye	7.1	9.5	13.3	15.2	17.2	18.6	19.2	19.6	21.6	21.4	25.2	23.7	26.5	...
Northern pike	10.2	15.6	19.4	22.2	24.6	26.5	28.9	32.7	33.4	38.7	39.6	42.0	48.0	...
Muskellunge	6.8	15.7	19.9	25.4	31.9	34.7	36.8	39.2	41.7	45.3	48.7	47.5	49.7	...
Smelt	...	5.3	6.9	7.7	8.1	8.8	9.6	...	...	...	...	...	...	...
Brook trout	3.0	6.4	9.0	11.5	15.1	18.8	21.3	23.9	...	...	...	...	...	...
Rainbow trout (inland lakes and streams)	2.2	6.3	8.4	10.3	11.0	...	...	...	...	...	...	...	...	...
Steelhead (lake-run rainbow)	...	13.4	17.0	18.7	23.6	25.4	28.1	30.0	30.4	...	...	...	...	...

[1]From Laarman (1964), Length of common Michigan sport fishes at successive ages, Michigan Fisheries No. 7, Department of Fisheries, School of Natural Resources, The University of Michigan, Ann Arbor, MI.

6.9 Annulus Formation Method

6.9.1 This technique is based on the fact that fish are poikilothermic animals and the rate at which their body processes function are affected by the temperature of the water in which they live. Growth is rapid during the warm season and slows greatly or stops in winter. This seasonal change produces a band (annulus) in such hard bony structures as scales, otoliths (ear stones), fin rays and spines, and vertebrae each year the fish lives. Scales (Figure 2) are most commonly used in determining the age and yearly rate of growth because they lengthen throughout the life of the fish at a predictable ratio to the annual increment in body length. The location of the body from where the scales are obtained is important. Each species of fish has a specific body area from which scales should be removed for optimum clarity and ease of identifying the annuli and a size at which scale formation begins (Jearld, 1983; Lagler, 1956; Weatherley, 1972). Coin envelopes are frequently used for holding scales and for recording field data (Figure 3).

Collection No. ____________________

Species ____________________

Location ____________________

Date ________ Time ________ Mile ________

Sampling Device ____________________

Collected by ____________________

S.L. ______ T.L. __________ Wt. __________

Sex ___ Maturity/and state of organs ____

____

Annuli ________ Condition ____________

Figure 3. Example of recording field data information of scale samples for age and growth studies.

6.9.2 Aging can be accomplished by use of a side-field, low-powered microscope, but a microprojector is preferred for determining the rate of growth. Computer assisted microprojectors have been developed for reading scales more rapidly and accurately.

6.9.3 It is important that the investigator realize that not all annuli-like markings are valid. "Spawning-checks", "false annuli", or other annuli-like marks may be present because of disease, body injury, spawning, etc.

6.9.4 The duration of sampling and the number of fish that must be collected are not as critical as the length-frequency method. Sampling can cover a considerable period and only a single method need be used for capturing the fish. Specialized equipment and trained personnel are needed, however, to identify, analyze, and interpret the data.

6.9.5 To determine any changes in the growth rate of a fish population, it is essential to use both the length-frequency and annulus methods and have samples from unaffected localities and/or sufficient background data from the sampling area. Any changes detected may be attributed to a single or a combination of natural or man-associated activities that altered the environment. Some of the most obvious natural modifications are a change in the average annual water temperature, fluctuating water levels, and availability of food. Man may also influence the water temperature and levels, physically alter the environment and fish habitat by damming or dredging activities, surface mining activities, and introducing substances that directly or indirectly affect the well-being of the fish population. It is evident, therefore, that it may be impossible to pin-point what or who was responsible for the change in the growth rate of a fish population except in a small lake.

6.10 Condition Factor (Coefficient of Condition)

6.10.1 The condition of fish can be estimated mathematically or by evaluating physical appearance.

6.10.2 Mathematically, the coefficient of condition is utilized to express the relative degree of well-being, robustness, plumpness or fatness of fish. It is based on a length-weight relationship and is calculated by the formula:

$$\text{Coefficient of Condition} \quad K_{TL} = \frac{W\,10^5}{L^3}$$

W = weight in grams
L = length in millimeters
10^5 = factor to bring the value of K near unity
TL = designation of measuring system used (fork, standard, or total length)

6.10.2.1 The coefficient of condition is "K" when the metric system is used in expressing the length and weight, and "C" when the English system is used.

6.10.3 The coefficient of condition has been used by ichthyologists and fishery biologists to determine the suitability of the environment for a species. However, it is not recommended for use in short term water quality studies because any non-environmental factors influence the values derived, e.g., changes due to age, sexual differences, and changes with seasons. These natural fluctuations make it extremely difficult to attribute any change to the quality of the water from which the fish are collected and must be taken into account when designing long term studies and evaluating data.

6.10.4 The observance of the physical appearance or condition of fish will usually indicate the general state of their well being and give some broad indication of the quality of their environment. When fish are captured they should be examined to see if they appear emaciated, are diseased, or contain parasites. The condition of their gills should also be checked. Healthy fish will be active when handled and are reasonably plump. Dissect a few specimens and check the internal organs for disease or parasites. The stomach of fish should also be examined to determine if the fish were actively feeding prior to capture.

6.10.5 For more detailed information on age, growth, and conditions of fish, see Anderson and Gutreuter (1983), Bagenal and Tesch (1978), Calhoun (1966), Carlander (1969), Everhart et al. (1975), Goede (1991), Jearld (1983), Lagler (1956), Lux (1971), Norman (1951), Ricker (1975), Schram et al. (1992), Summerfelt (1987), and Weatherley (1972).

6.11 Relative Weight Index

6.11.1 Usefulness of typical fisheries metrics for evaluating sensitive indicator organisms at the population level provide useful information in comparing subtle differences between sites. The drawbacks to using standard fisheries approaches are the limitations of either state developed or regional expectations and the lack of resolution linked with causes. The assessments require a large sample for site comparison and a large number of reference stations for determining the expected population regression line. The traditional approach to the assessment of condition involves the use of a Fulton-type (Anderson and Guetreuter, 1983) condition factor. This is calculated as:

$$K = W/L^3$$

where W is weight (g) and L is length (mm). These factors are both length and species dependent. Therefore, it is improper to compare fish of different species or fish of the same species at different lengths. Le Cren (1951) developed the relative condition factor:

$$K = W/W' \times 100$$

where W is the observed weight and W′ is the length specific expected weight for fish in the populations under study as predicted by a weight-length regression equation calculated for that population. This approach solved the problem of comparing fish of different lengths and species but, because a different weight-length regression was calculated for each population, interpopulational comparisons were not possible. The relative weight (W_r) index (Wege and Andrson, 1978) enabled interpopulational comparisons by making the standard weight-length (W_s) regression species-specific rather than population specific or location specific. Relative weight is calculated as:

$$W_r = W/W_s \times 100$$

where W_s is the length-specific standard weight predicted by a weight-length regression constructed to represent the species as a whole.

6.11.2 W_s equations have been defined in most cases to represent populations in better than average conditions (reference conditions) based on the assumption that attempting to produce fish populations that attain only average condition generally does not represent a typical management goal. W_s should be considered a benchmark for comparison of samples and populations. Comparisons are based on the 75th percentile of the weight. An alternative technique, regression-line-percentile (RLP), is based on comparison of $\log_{10}$weight-$\log_{10}$length regression equations for each population whereas the typical W_r equation is based on pooled length-weight data.

6.11.3 Murphy et al. (1991) discussed the development of the index and expounded upon the status and W_r regression equation for 27 species. To calculate W_r properly requires data from representative or reference stations over a broad range for the species of interest. Slopes of less than 3.0 are considered inappropriate for most species because such a slope indicates the species becomes thinner with increased length. Low slopes may also results from including small fish in the regression. Differences of weighing small fishes and the inherent problems of weighing small fishes in the field may preclude development of a single equation for an entire species life history. A minimum applicable length is used to determine the minimum size which should be weighed. For other species the minimum length is a function of the variance:mean ration for $\log_{10}$ weight where it sharply increased.

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